

We claim:

1. An automated method for identifying compounds that induce internalization of cell surface receptor proteins comprising

5        -providing an array of locations containing multiple cells to be treated with a test compound, wherein the cells possess a cell surface receptor protein of interest, and wherein the cell surface receptor protein is either expressed as a luminescently labeled protein, or is luminescently labeled by contacting the cell with a luminescently labeled molecule that binds to the cell surface receptor of interest, wherein the contacting can be carried out  
10 either before or after treatment with the test compound;

      -treating the cells with the test compound;

      -scanning multiple cells in each of the locations containing multiple cells to obtain luminescent signals from the luminescently-labeled cell surface receptor protein;

      -converting the luminescent signals into digital data; and

15        -utilizing the digital data to automatically determine whether the test compound has induced internalization of the luminescently labeled cell surface receptor protein.

2. The method of claim 1 further comprising determining a number of cells that have internalized the luminescently labeled cell surface receptor protein.

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3. The method of claim 2 further comprising determining a total cell number.

4. The method of claim 3 wherein the determining of the total cell number comprises the steps of:

- a. acquiring an image of the cell nuclei;
- b. segmenting the image of the cell nuclei; and
- 5 c, calculating the total area of all the nuclei in the image of the cell nuclei.

5. The method of claim 2 wherein the determining a number of cells that have internalized the luminescently labeled cell surface receptor protein comprises the steps of:

- a. acquiring an object image of the luminescently labeled cell surface receptor
- 10 protein in or on the cells;
- b. segmenting the object image; and
- c. determining whether objects in the segmented object image represent valid
- internalized luminescently labeled cell surface receptor proteins.

15 6. The method of claim 5 further comprising at least one of the following:

- a. removing artifacts from the object image; or
- b. correcting for background luminescence.

7. The method of claim 5 further comprising measuring at least one of the following:

- 20 (a) a number of objects that were determined to represent valid internalized cell surface receptors;

(b) an aggregate area of the objects that were determined to represent valid internalized cell surface receptors;

(c) an aggregate intensity of the objects that were determined to represent valid internalized cell surface receptors; or

5 (d) a normalized aggregate intensity of the objects that were determined to represent valid internalized cell surface receptors.

8. The method of claim 1 wherein sub-regions of the array of locations containing multiple cells are sampled multiple times at intervals to provide kinetic measurement of  
10 cell surface receptor protein internalization into the cell.

9. A method for identifying compounds that inhibit internalization of cell surface receptor proteins comprising

-providing an array of locations containing multiple cells to be treated with a test  
15 compound, wherein the cells possess a cell surface receptor protein of interest, and wherein the cell surface receptor protein is either expressed as a luminescently labeled protein, or is luminescently labeled by contacting the cell with a luminescently labeled molecule that binds to the cell surface receptor of interest, wherein the contacting can be carried out either before or after treatment with the test compound;

20 -treating the cells with a test compound;

-treating the cells with a ligand that causes the cell surface receptor protein to be internalized in the absence of the test compound;

-scanning multiple cells in each of the locations containing cells to obtain  
luminescent signals from the luminescently labeled receptor protein;

-converting the luminescent signals into digital data; and

-utilizing the digital data to determine whether the test compound has inhibited  
5 ligand-induced internalization of the luminescently labeled cell surface receptor protein  
into the cell.

10. The method of claim 9 further comprising determining a number of cells that have  
internalized the luminescently labeled cell surface receptor protein.

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11. The method of claim 10 further comprising determining a total cell number.

12. The method of claim 11 wherein the determining of the total cell number comprises  
the steps of:

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- a. acquiring an image of the cell nuclei;
  - b. segmenting the image of the cell nuclei; and
  - c, calculating the total area of all the nuclei in the image of the cell nuclei.

13. The method of claim 10 wherein the determining a number of cells that have  
20 internalized the luminescently labeled receptor protein comprises the steps of:

- a. acquiring an object image of the luminescently labeled cell surface receptor  
protein in or on the cells;

b. segmenting the object image; and  
c. determining whether objects in the segmented object image represent valid internalized luminescently labeled cell surface receptor proteins.

5 14. The method of claim 13 further comprising at least one of the following:

- a. removing artifacts from the object image; or
- b. correcting for background luminescence.

15. The method of claim 13 further comprising measuring at least one of the following:

- 10 (a) a number of objects that were determined to represent valid internalized cell surface receptors;
- (b) an aggregate area of the objects that were determined to represent valid internalized cell surface receptors;
- (c) an aggregate intensity of the objects that were determined to represent valid internalized cell surface receptors; or
- 15 (d) a normalized aggregate intensity of the objects that were determined to represent valid internalized cell surface receptors.

16. The method of claim 9 wherein sub-regions of the array of locations containing  
20 multiple cells are sampled multiple times at intervals to provide kinetic measurement of the inhibition of cell surface receptor protein internalization into cell.

17. A method for identifying compounds that induce internalization of cell surface receptor proteins comprising

-providing an array of locations containing multiple cells to be treated with a test compound, wherein the cells possess a cell surface receptor protein of interest, and wherein the cell surface receptor protein is either expressed as a luminescently labeled protein, or is  
5 luminescently labeled by contacting the cell with a luminescently labeled molecule that binds to the cell surface receptor of interest, wherein the contacting can be carried out either before or after treatment with the test compound;

-treating the cells with an indicator that produces a detectable signal upon  
10 stimulation of the receptor protein

-treating the cells with a test compound;

-scanning the cells in a high throughput mode to identify those cells that exhibit the detectable signal;

-selectively scanning only a subset of the cells in a high content mode to obtain  
15 luminescent signals from the luminescently labeled receptor protein, wherein the subset consists of the cells that exhibit the detectable signal during scanning in the high throughput mode;

-converting the luminescent signals into digital data; and

-utilizing the digital data to determine whether the test compound has induced  
20 internalization of the luminescently labeled cell surface receptor into the cell.

18. The method of claim 17 further comprising determining a number of cells that have internalized the luminescently labeled cell surface receptor protein.

19. The method of claim 18 further comprising determining a total cell number.

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20. The method of claim 19 wherein the determining of the total cell number comprises the steps of:

- a. acquiring an image of the cell nuclei;
- b. segmenting the image of the cell nuclei; and
- 10 c. calculating the total area of all the nuclei in the image of the cell nuclei.

21. The method of claim 18 wherein the determining a number of cells that have internalized the luminescently labeled receptor protein comprises the steps of:

- a. acquiring an object image of the luminescently labeled cell surface receptor  
15 protein in or on the cells;
- b. segmenting the object image; and
- c. determining whether objects in the segmented object image represent valid  
internalized luminescently labeled cell surface receptor proteins.

20 22. The method of claim 21 further comprising at least one of the following:

- a. removing artifacts from the object image; or
- b. correcting for background luminescence.

23. The method of claim 21 further comprising measuring at least one of the following:

(a) a number of objects that were determined to represent valid internalized cell surface receptors;

5 (b) an aggregate area of the objects that were determined to represent valid internalized cell surface receptors;

(c) an aggregate intensity of the objects that were determined to represent valid internalized cell surface receptors; or

10 (d) a normalized aggregate intensity of the objects that were determined to represent valid internalized cell surface receptors.

24. The method of claim 17 wherein sub-regions of the array of locations containing multiple cells are sampled multiple times at intervals to provide kinetic measurement of cell surface receptor internalization.

15 25. A method for identifying compounds that inhibit internalization of cell surface receptor proteins comprising

-providing an array of locations containing multiple cells to be treated with a test compound, wherein the cells possess a cell surface receptor protein of interest, and wherein  
20 the cell surface receptor protein is either expressed as a luminescently labeled protein, or is luminescently labeled by contacting the cell with a luminescently labeled molecule that binds to the cell surface receptor of interest, wherein the contacting can be carried out



either before or after treatment with the test compound;

- treating the cells with an indicator that produces a detectable signal upon stimulation of the receptor protein

- treating the cells with a test compound;

5        -treating the cells with a ligand that causes the cell surface receptor protein to be internalized into the cell in the absence of the test compound;

- scanning the cells in a high throughput mode to identify those cells that do not exhibit the indicator-induced detectable signal;

- selectively scanning only a subset of the cells in a high content mode to obtain

10        luminescent signals from the luminescently labeled receptor protein, wherein the subset consists of the cells that did not exhibit the desired detectable signal during scanning in the high throughput mode;

- converting the luminescent signals into digital data; and

- utilizing the digital data to determine whether the test compound has inhibited

15        ligand-induced internalization of the luminescently labeled cell surface receptor protein into the cell.

26.        The method of claim 25 further comprising determining a number of cells that have internalized the luminescently labeled cell surface receptor protein.

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27.        The method of claim 26 further comprising determining a total cell number.

28. The method of claim 27 wherein the determining of the cell number comprises the steps of:

- a. acquiring an image of the cell nuclei;
- b. segmenting the image of the cell nuclei; and
- 5 c, calculating the total area of all the nuclei in the image of the cell nuclei.

29. The method of claim 26 wherein the determining a number of cells that have internalized the luminescently labeled receptor protein comprises the steps of:

- a. acquiring an object image of the luminescently labeled cell surface receptor  
10 protein in or on the cells;
- b. segmenting the object image; and
- c. determining whether objects in the segmented object image represent valid  
internalized luminescently labeled cell surface receptor proteins.

15 30. The method of claim 29 further comprising at least one of the following:

- a. removing artifacts from the object image; and
- b. correcting for background luminescence.

31. The method of claim 29 further comprising measuring at least one of the following:

- 20 (a) a number of objects that were determined to represent valid internalized  
cell surface receptors;

(b) an aggregate area of the objects that were determined to represent valid internalized cell surface receptors;

(c) an aggregate intensity of the objects that were determined to represent valid internalized cell surface receptors; or

5 (d) a normalized aggregate intensity of the objects that were determined to represent valid internalized cell surface receptors.

32. The method of claim 25 wherein sub-regions of the array of locations containing multiple cells are sampled multiple times at intervals to provide kinetic measurement of  
10 cell surface receptor internalization.

33. A computer readable storage medium comprising a program containing a set of instructions for causing a cell screening system to execute the method of claim 1 wherein the cell screening system comprises an optical system with a stage adapted for holding a  
15 plate containing cells, a means for moving the stage or the optical system, a digital camera, a means for directing light emitted from the cells to the digital camera, and a computer means for receiving and processing the digital data from the digital camera.

34. A computer readable storage medium comprising a program containing a set of  
20 instructions for causing a cell screening system to execute the method of claim 9 wherein the cell screening system comprises an optical system with a stage adapted for holding a plate containing cells, a means for moving the stage or the optical system, a digital camera,

a means for directing light emitted from the cells to the digital camera, and a computer means for receiving and processing the digital data from the digital camera.

35. A computer readable storage medium comprising a program containing a set of  
5 instructions for causing a cell screening system to execute the method of claim 17 wherein the cell screening system comprises an optical system with a stage adapted for holding a plate containing cells, a means for moving the stage or the optical system, a digital camera, a means for directing light emitted from the cells to the digital camera, and a computer means for receiving and processing the digital data from the digital camera.

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36. A computer readable storage medium comprising a program containing a set of instructions for causing a cell screening system to execute the method of claim 25 wherein the cell screening system comprises an optical system with a stage adapted for holding a plate containing cells, a means for moving the stage or the optical system, a digital camera,  
15 a means for directing light emitted from the cells to the digital camera, and a computer means for receiving and processing the digital data from the digital camera.

37. A kit for identifying compounds that induce or inhibit internalization of cell surface receptor proteins comprising:

20 (a) at least one antibody or fragment thereof that specifically binds to a cell surface receptor protein of interest; and

(b) instructions for using the antibody to identify compounds that induce or inhibit internalization of the cell surface receptor protein of interest into cells.

38. The kit of claim 37 wherein the cell surface receptor protein of interest is a G-  
5 protein coupled receptor.

39. The kit of claim 38 wherein the G-protein coupled receptor is parathyroid hormone  
receptor.